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Microcirculatory Changes as a Hallmark of Aging in the Heart and Kidney

Cambios Microcirculatorios como Sello Distintivo del Envejecimiento en el Corazón y Riñón

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SUMMARY: Changes in the microcirculation of multiple tissues and organs have been implicated as a possible mechanism in physiological aging. In particular, vascular endothelial growth factor is a secretory protein responsible for regulating angiogenesis via altering endothelial proliferation, survival, migration, extracellular matrix degradation and cell permeability. The aim of the present study was to evaluate the role of vascular endothelial growth factor in the progression of morphological alterations caused by physiological aging in the heart and kidney and to examine its relation to changes in capillary density. We used two age groups of healthy Wistar rats – 6- and 12-month-old. The expression of vascular endothelial growth factor was examined through immunohistochemistry and immunofluorescence and assessed semi-quantitatively. Changes in capillary density were evaluated statistically and correlated with the expression of vascular endothelial growth factor. We reported stronger immunoreactivity for vascular endothelial growth factor in the left compared to the right ventricle and also observed an increase in its expression in both ventricles in older animals. Contrasting results were reported for the renal cortex and medulla. Capillary density decreased statistically in all examined structures as aging progressed. The studied correlations were statistically significant in the two ventricles in 12-month-old animals and in the renal cortex of both age groups. Our results shed light on some changes in the microcirculation that take place as aging advances and likely contribute to impairment in the function of the examined organs.

KEY WORDS: Vascular endothelial growth factor (VEGF); Capillary density (CD); Myocardium; Kidney; Aging.

INTRODUCTION

Biological aging is a physiological process driven by various factors, including genetics, gender, environment and nutrition. Inevitably, it leads to morphological and physiological changes in all cells, tissues and organs. Therefore, older age becomes a major risk factor for the development of a wide array of pathological conditions in different organs and systems, including the cardiovascular and urinary systems (Chiao & Rabinovitch, 2015; Denic *et al*., 2017). One of the changes occurring as a consequence to aging is a decrease in capillary density (CD) which alters the regenerative capacity and functionality of the affected tissues (Iliev *et al*., 2017). Vascular endothelial growth factor (VEGF) is a secretory protein responsible for regulating angiogenesis via altering endothelial proliferation, survival, migration, extracellular matrix degradation and cell permeability (Ferrara, 2004). Five different subtypes of VEGF have been isolated in humans: VEGF-A; VEGF-B; VEGF-C; VEGF-D, and placental growth factor. Out of those five variants, VEGF-A (widely referred to simply as VEGF) is the most widely studied so far and serves as the main cytokine regulating neovascularization (Braile *et al*., 2020).

Cardiovascular diseases are the leading cause of death globally and aging represents one of the most important risk factors in the development of such disorders (Chiao & Rabinovitch, 2015). Advancing age results in a decrease of myocardial regenerative capacity and resistance to injury, followed by an array of pathological alterations, including, but not limited to, atrial fibrillation, diastolic dysfunction and hypertrophy (Huynh *et al*., 2012; Chiao & Rabinovitch, 2015).

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The hallmark of cardiac aging is the development of reactive interstitial fibrosis, causing an increase in cardiac interstitial space without significant loss of cardiomyocytes (Biernacka & Frangogiannis, 2011). Other authors have also described a decrease in CD in areas with more prominent fibrosis, proposing the lack of neovascularization as a crucial factor for the development and progression of fibrosis (Xiao *et al*., 2018).

Cardiomyocytes express two different receptors that bind VEGF-A: VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2), through which VEGF promotes morphogenesis, contractility and regeneration in the cardiomyocytes (Dobbin *et al*., 2021). Furthermore, cardiomyocytes have the potential to produce and release VEGF under mechanical stress, inflammation or cytokine stimulation (Braile *et al*., 2020). That intrinsic VEGF production by cardiomyocytes has been associated with a worse prognosis and disease severity (Braile *et al*., 2020).

Over the course of aging, kidneys also undergo a series of alterations including loss of tubules, development of glomerulosclerosis and progressive interstitial fibrosis (Zhou *et al*., 2008; Denic *et al*., 2017). Kidneys are highly vascularised organs with abundance of glomerular and peritubular capillaries and the decrease in CD may cause morphological and physiological changes leading to chronic kidney disease (Chade & Hall, 2016). VEGF, as the primary cytokine regulating angiogenesis, possibly plays a role in renal aging by altering the maintenance of vascular networks and neovascularization (Chade & Hall, 2016). In addition, kidneys are both a major source and a target of VEGF action – podocytes and tubular epithelial cells produce VEGF; on the other hand, podocytes and endothelial cells both express VEGFR1 and VEGFR2 (Chade & Hall, 2016). These seemingly opposing relations only serve to underscore the complex role that VEGF plays in regulating angiogenesis under both physiological and pathological conditions.

Therefore, the aim of the present study was to evaluate the role of VEGF in the progression of morphological alterations caused by physiological aging. Because VEGF serves as a potent factor for angiogenesis, herein we conducted a comparative study of the expression of VEGF and CD in heart and kidney of two age groups of Wistar rats (WR).

MATERIAL AND METHOD

Experimental animals. Two age groups of WR were used for the purposes of the present study – 6-month-old (young) and 12-month-old (adult) (Stamenov *et al*., 2022). Each group comprised twelve male rats randomly selected from a large population of WR in the Laboratory of the Department of Anatomy, Histology and Embryology at the Medical University of Sofia, Bulgaria. All animal procedures were conducted in the manner previously described (Stamenov *et al*., 2022).

Tissue preparation

Heart. Six randomly selected rats from each group were sacrificed for myocardial specimens according to the previously described standardised procedure (Stamenov *et al*., 2022).

Kidney. Six randomly selected rats from each group were sacrificed and their kidneys were obtained for analysis according to the previously described standardised procedure (Stamenov *et al*., 2022).

Light microscopy. Slides from the heart and kidney were processed for routine light microscopic study in the manner previously described (Stamenov *et al*., 2022).

Immunohistochemistry. The immunohistochemical study was conducted through the heat-induced epitope retrieval (HIER) technique as previously described (Stamenov *et al*., 2022). We used mouse monoclonal anti-VEGF-A IgG antibody (Santa Cruz Biotechnology Catalogue No. sc-7269, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) at concentration 1:250. Further, incubation with mouse IgG kappa binding protein (m-IgGk BP) conjugated to horseradish peroxidase (HRP) (Santa Cruz Biotechnology Catalogue No. sc-516102) at concentration 1:75 for 2 h was performed. All other procedures followed the standardised protocol described in our previous work (Stamenov *et al*., 2022).

Semi-quantitative analysis of VEGF expression. The protocol for the semi-quantitative analysis of the expression of VEGF followed the standardised procedure described in our previous works (Stamenov *et al*., 2022).

Immunofluorescence. Paraffin-embedded sections were dewaxed using xylene and rehydrated through usage of ascending series of alcohols, with final rehydration steps using H_2O. Slides were put in 10 mM sodium citrate buffer (pH 6.0) for HIER technique to recover antigens. Slides were incubated overnight at 4 °C with the monoclonal anti-VEGF-A IgG antibody (Santa Cruz Biotechnology Catalogue No. sc-7269) at concentration 1:250. The slides were rinsed in PBS (Merck Catalogue No. 6505-4 L, Merck KGgA, Darmstadt, Germany) and then treated with fluorescently labelled secondary antibody: a green fluorescent dye for

VEGF-A (m-IgGk BP) conjugated to CruzFluor™ 488 (CFL 488, Santa Cruz Biotechnology Catalogue No. sc-516176) at a dilution of 1:250 in the dark chamber for 1h. After rinsing the slides with PBS (Merck Catalogue No. 6505-4 L), cover slips were mounted with a hard-set mounting medium.

Morphometric analysis of CD. The morphometric analysis was performed on H&E-stained slides from each organ of each animal. Quantitative data were obtained with a computerised system for image analysis NIS-Elements Advanced Research (Ver. 2.30). CD was quantified according to well-established protocols (Ward *et al*., 2003; Sun *et al*., 2018).

Statistical analysis. The statistical analysis was performed with the SPSS software (IBM Corporation, Armonk, New York, United States). Data distribution was not normal, as assessed through the Kolmogorov–Smirnov test. The Mann-Whitney test was then used to test for statistically significant differences between CD at age 6 and 12 months in the left ventricle (LV), right ventricle (RV), renal cortex (RC) and renal medulla (RM). Spearman's correlation was used to test whether a correlation exists between CD per slide and VEGF (expressed as the total score of the examined area on the slide). A standard level of significance a (p value) = 0.05 was used in all tests.

RESULTS

Immunohistochemical analysis and semi-quantitative analysis of VEGF expression

The reported VEGF immunoreactivity was described in the cytoplasm of cardiomyocytes, in the walls of blood vessels of various calibres and in the perivascular zones. Expression in the LV was stronger than in the RV in both age groups and was noted to increase in older animals (Fig. 1). Immunoreactivity in the RC was reported in the visceral layer of Bowman's capsule, as well as in epithelial cells lining the lumen of proximal and distal tubular segments, while no staining was observed in glomerular capillary tufts in both age groups (Figs. 2A and 2B). Interestingly, contrary to the myocardium, VEGF expression in the RC decreased in older animals. In the RM, VEGF expression was mainly observed in the collecting ducts and in epithelial cells of the loops of Henle and remained unchanged in 12-month-old WR compared to the younger group (Figs. 2C and 2D).

The results of the semi-quantitative analysis of the intensity of immunoreactivity of VEGF are shown in Table I. VEGF expression in the LV of 6-month-old WR was low

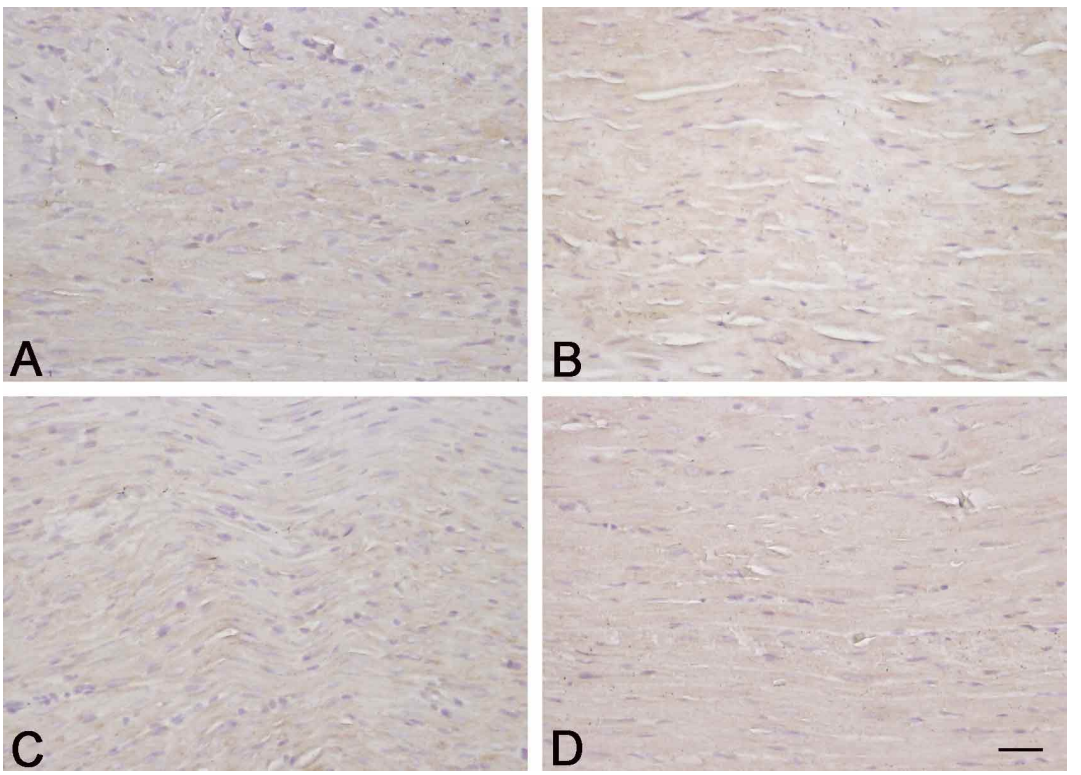

Fig. 1. Immunohistochemical staining for vascular endothelial growth factor (VEGF) in the myocardium of Wistar rats (WR). Size bar – 50 μm. A. Left ventricle (LV), 6-month-old WR; B. Left ventricle (LV), 12-month-old WR; C. Right ventricle (RV), 6-month-old WR; D. Right ventricle (RV), 12-month-old WR.

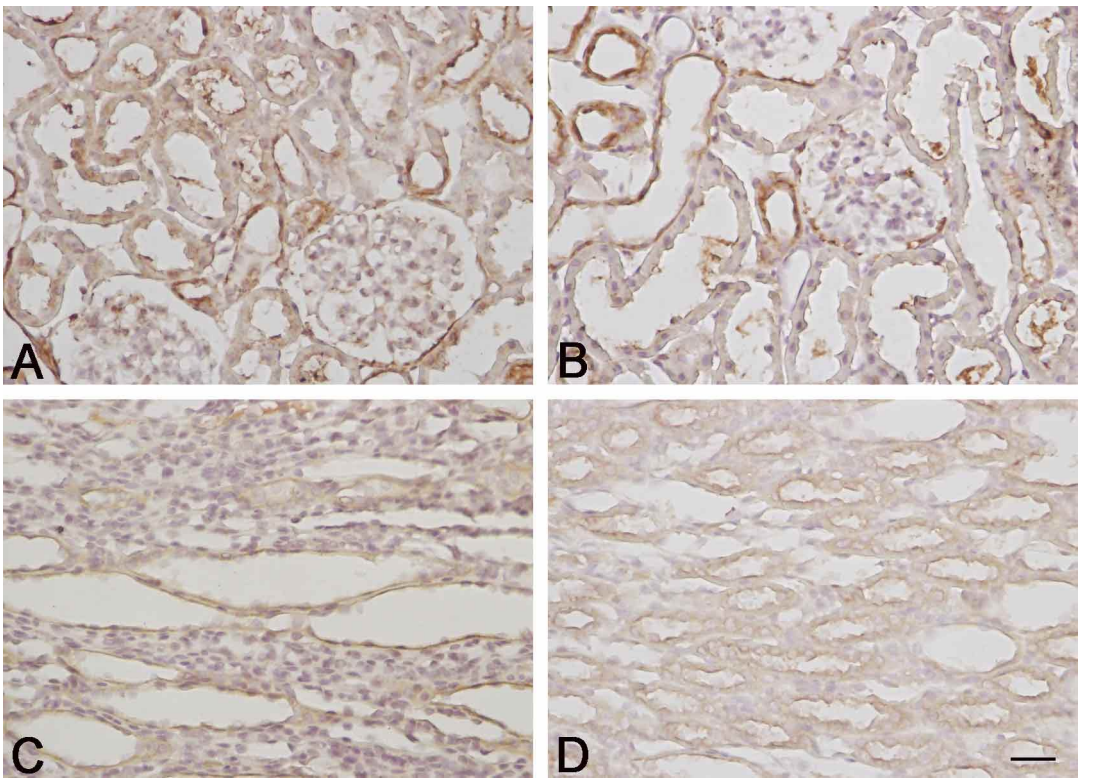

Fig. 2. Immunohistochemical staining for vascular endothelial growth factor (VEGF) in the kidney of Wistar rats (WR). Size bar – 50 μm. A. Renal cortex (RC), 6-month-old WR; B. Renal cortex (RC), 12-month-old WR; C. Renal medulla (RM), 6-month-old WR; D. Renal medulla (RM), 12-month-old WR.

Table I. Semi-quantitative analysis of the intensity of immunohistochemical staining for vascular endothelial growth factor in the myocardium of the left and right ventricle and in the renal cortex and medulla in 6- and 12-month-old Wistar rats.

VEGF	LV	RV	RC	RM
6-month-old WR	High-positive (3+) (0%) Positive (2+) (3%) Low-positive (1+) (40%) Negative (0) (57%)	High-positive (3+) (0%) Positive (2+) (0%) Low-positive (1+) (16%) Negative (0) (84%)	High-positive (3+) (0%) Positive (2+) (69%) Low-positive (1+) (21%) Negative (0) (10%)	High-positive (3+) (0%) Positive (2+) (7%) Low-positive (1+) (42%) Negative (0) (51%)
12-month-old WR	High-positive (3+) (0%) Positive (2+) (5%) Low-positive (1+) (72%) Negative (0) (23%)	High-positive (3+) (0%) Positive (2+) (3%) Low-positive (1+) (47%) Negative (0) (50%)	High-positive (3+) (0%) Positive (2+) (36%) Low-positive (1+) (56%) Negative (0) (8%)	High-positive (3+) (0%) Positive (2+) (4%) Low-positive (1+) (45%) Negative (0) (51%)

The percentage of each score indicates the percentage of visual fields that the IHC Profiler assigned this score to. LV – left ventricle; RV – right ventricle; RC – renal cortex; RM – renal medulla; WR – Wistar rats; VEGF – vascular endothelial growth factor.

positive (1+) in 40% and negative (0) in nearly half of the examined fields, whereas in the 12-month-old animals the predominant expression was low-positive (1+), while almost third of the fields stained negatively (0). The tendency of the intensity of immunoreactivity of VEGF noted in the RV was mainly negative (0), while in 12-month-old WR, nearly half the fields were low-positive (1+) and the other half – negative (0). The RC of younger animals was characterised by mostly positive (2+) expression, while in the older group just over half of the fields were low-positive (1+) and 36%

from the observed fields were positive (2+). Interestingly, in the RM of both groups of animals the expression was similar, with almost equal ratio between negative (0) and low-positive (1+) intensity of immunoreactivity of VEGF, as predominant was negative (0) expression – in about 50% of the examined fields.

Immunofluorescence

Immunofluorescence for VEGF in the myocardium was reported inside the cytoplasm of cardiac muscle cells, particularly the perinuclear areas, as well in perivascular zones (Fig. 3). The intensity of the reaction was notably stronger in the LV compared to the RV and suggested a stronger VEGF expression in 12-month-old WR.

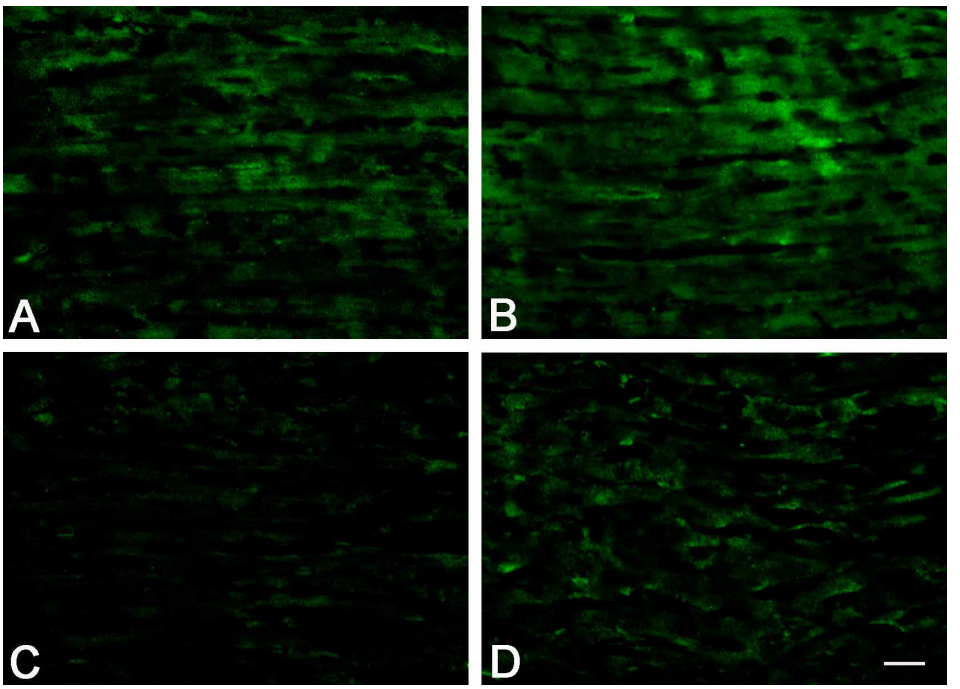

Fig. 3. Immunofluorescent labelling of vascular endothelial growth factor (VEGF) in the myocardium of Wistar rats (WR). Size bar – 30 μm. A. Left ventricle (LV), 6-month-old WR; B. Left ventricle (LV), 12-month-old WR; C. Right ventricle (RV), 6-month-old WR; D. Right ventricle (RV), 12-month-old WR.

VEGF staining in the RC was observed in the visceral layer of Bowman's capsule and in the perinuclear area of epithelial cells of the renal tubules and appeared less strong in older animals (Figs. 4A and 4B). Similar findings were noted in the RM, where immunofluorescence for VEGF was reported in the perinuclear area of epithelial cells lining the lumens of collecting ducts and again appeared weaker in 12-month-old animals (Figs. 4C and 4D).

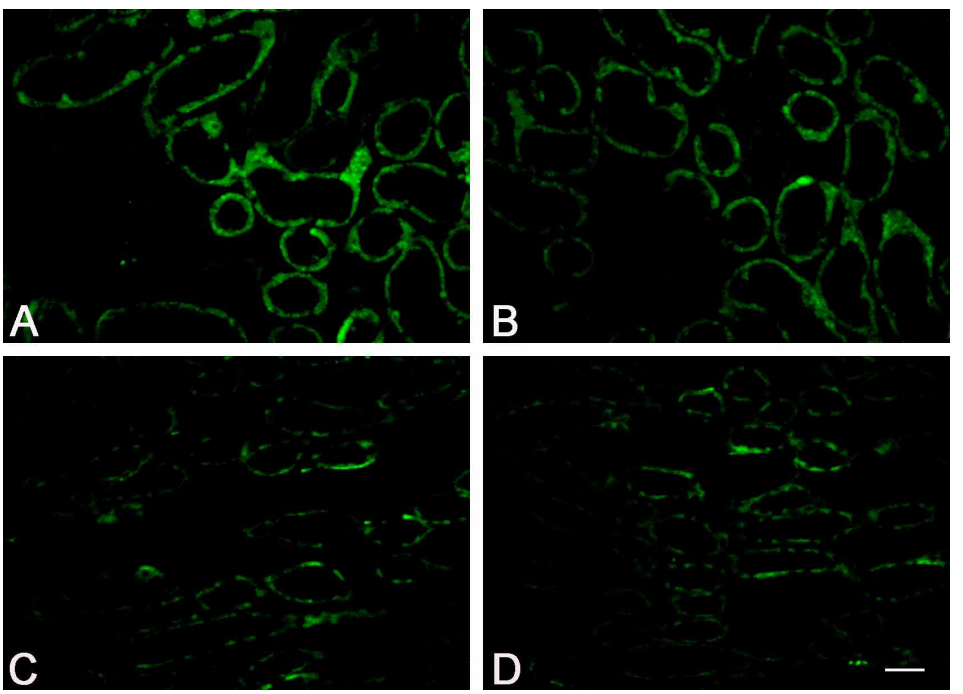

Fig. 4. Immunofluorescent labelling of vascular endothelial growth factor (VEGF) in the kidney of Wistar rats (WR). Size bar – 50 μm. A. Renal cortex (RC), 6-month-old WR; B. Renal cortex (RC), 12-month-old WR; C. Renal medulla (RM), 6-month-old WR; D. Renal medulla (RM), 12-month-old WR.

Comparative analysis of CD in 6- and 12-month-old WR

CD in the myocardium, RC and RM was evaluated on sections stained with H&E. Capillaries were observed as oval or circular structures with a lumen, sometimes filled with red blood cells and an endothelial cell lining the lumen.

Highest CD was reported for the LV, followed by the RV, while the lowest CD was in the RC and slightly higher values were reported in the RM in both age groups. A marked decrease in CD in 12-month-old WR compared to younger animals was noted in the myocardium of both ventricles, while in the RC and RM the change in the studied indicator was less abrupt. The conducted statistical analysis confirmed that decrease to be statistically significant (Table II; Fig. 5).

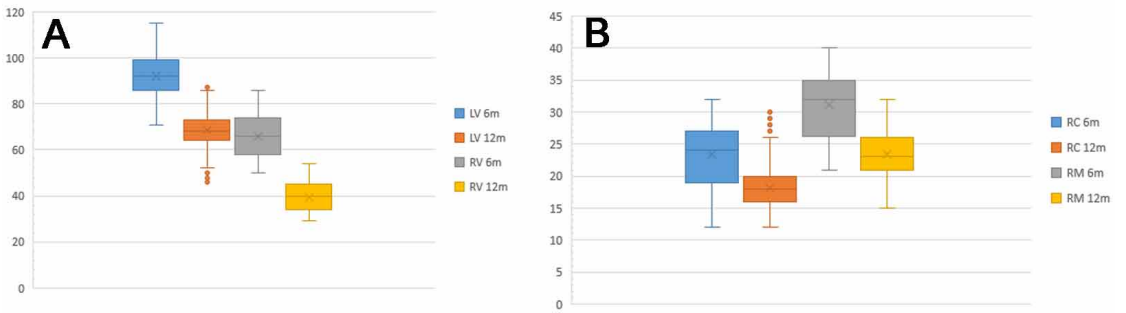

Fig. 5. Box and whisker plots showing the median (square), surrounded by a 'box', the vertical edge of which is the interval between the lower and upper quartile [25 %-75 %]. 'Whiskers' originating from this 'box' represent the non-outlier range. Circles – outliers. 6m – 6-month-old Wistar rats (WR); 12m – 12-month-old WR; LV – left ventricle; RV – right ventricle; RC – renal cortex; RM – renal medulla. A. Myocardium; B. Kidney.

Table II. Descriptive statistics for the parameter capillary density per high-power field on hematoxylin and eosin-stained slides in the heart and kidney of 6- and 12-month-old Wistar rats.

Capillary density	N	6-month-old WR					12-month-old WR					p-value
		Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	
LV	300	92.2	8.5	92	71	115	68.7	8.2	68	46	88	p<0.05
RV	300	65.8	9.4	66	50	86	39.4	6.4	40	29	54	p<0.05
RC	300	23.4	4.7	24	12	32	18.1	3.5	18	12	30	p<0.05
RM	300	31.2	5.0	32	21	40	23.3	4.0	23	15	32	p<0.05

LV – left ventricle; RV – right ventricle; RC – renal cortex; RM – renal medulla; WR – Wistar rats; N – number of high-power fields; SD – standard deviation.

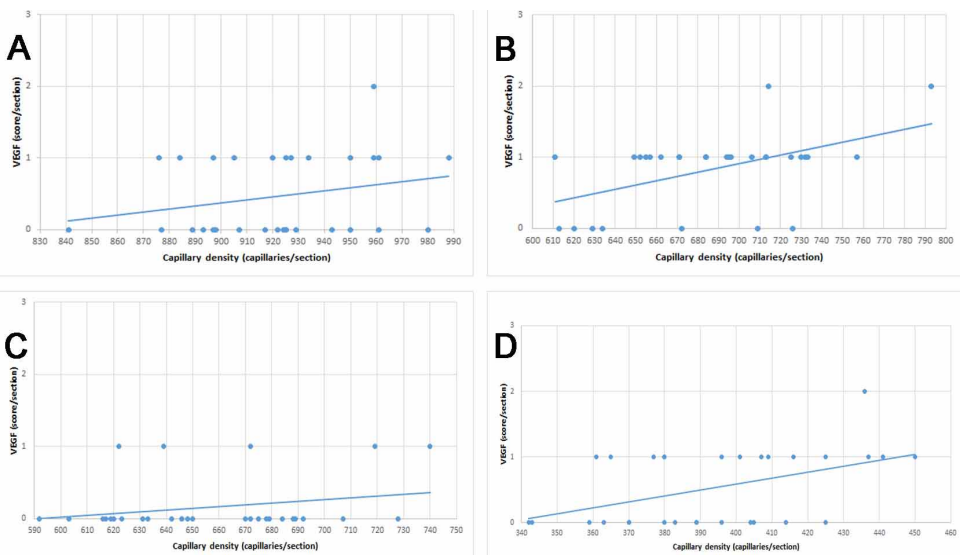

Fig. 6. Correlation between the immunohistochemical expression of vascular endothelial growth factor (VEGF) and capillary density (CD) in the myocardium of 6- and 12-month-old Wistar rats (WR). x-axis – CD expressed as total number of capillaries relative to the whole examined area on the slide; y-axis – VEGF expressed as total semi-quantitative score of the whole examined area on the slide. A. Left ventricle (LV), 6-month-old WR (r_s = 0.2117, p = 0.26143); B. Left ventricle (LV), 12-month-old WR (r_s = 0.4235, p = 0.0197); C. Right ventricle (RV), 6-month-old WR (r_s = 0.17571, p = 0.35301); D. Right ventricle (RV), 12-month-old WR (r_s = 0.40693, p = 0.02563).

Correlations between CD and VEGF expression

In all examined structures, a positive correlation was established between the CD per section and the expression of VEGF, assessed semi-quantitatively as score per section. In the group of young animals, we did not establish a statistically significant difference in the two ventricles (rs = 0.2117, p = 0.26143 in LV and r_s = 0.17571, p = 0.35301 in RV, respectively) (Figs. 6A and 6C), however a statistically significant correlation was observed in both LV (r_s = 0.4235, p = 0.0197) and RV (r_s = 0.40693, p = 0.02563) of adult WR (Figs. 6B and 6D). In the RC, the positive correlation was statistically significant in both age groups (r_s = 0.63746, p = 0.00015 in 6-month-old WR and r_s = 0.39134, p = 0.03248 in 12-month-old WR, respectively) (Figs. 7A and 7B). On the contrary, the correlation in the RM was found not to be statistically significant in young, as well as adult animals (r_s = 0.27025, p = 0.14863 in 6-month-old WR and r_s = 0.32974, p = 0.07516 in 12-month-old WR, respectively) (Figs. 7C and 7D).

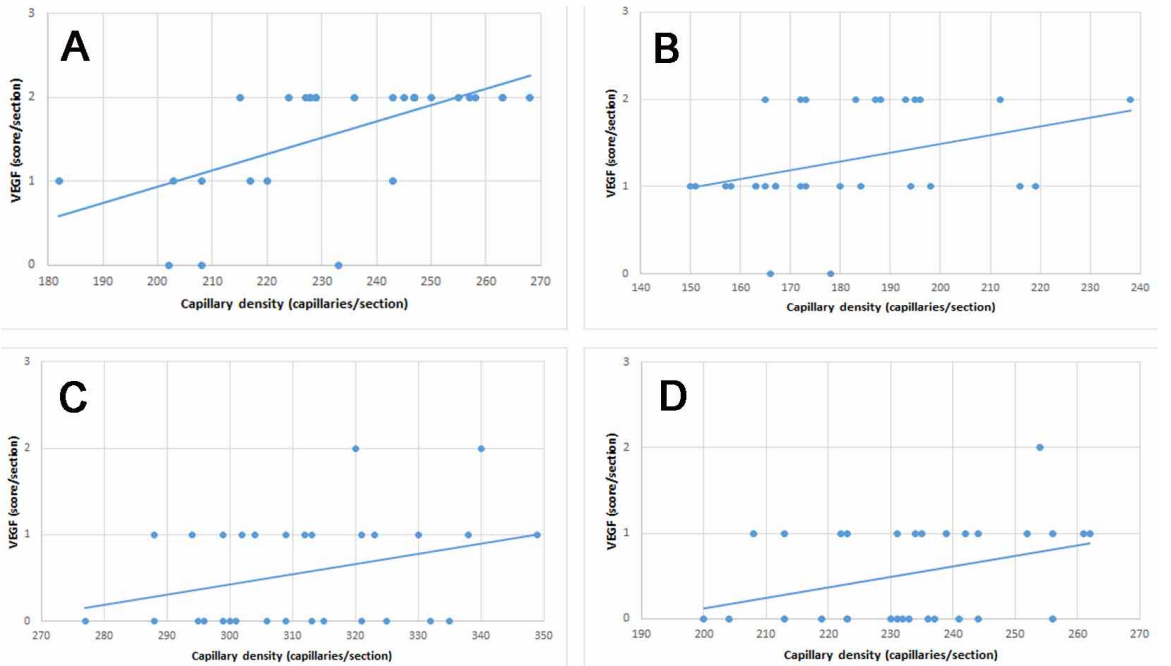

Fig. 7. Correlation between the immunohistochemical expression of vascular endothelial growth factor (VEGF) and capillary density (CD) in the kidney of 6- and 12-month-old Wistar rats (WR). x-axis – CD expressed as total number of capillaries relative to the whole examined area on the slide; y-axis – VEGF expressed as total semi-quantitative score of the whole examined area on the slide. A. Renal cortex (RC), 6-month-old WR (r_s = 0.63746, p = 0.00015); B. Renal cortex (RC), 12-month-old WR (r_s = 0.39134, p = 0.03248); C. Renal medulla (RM), 6-month-old WR (r_s = 0.27025, p = 0.14863); D. Renal medulla (RM), 12-month-old WR (r_s = 0.32974, p = 0.07516).

DISCUSSION

Our study strives to shed light on a complex yet essential adaptive mechanism – angiogenesis in the heart and kidney and its downregulation as a hallmark of age-related alterations. To the best of our knowledge, the present study is the first to focus particularly on age-related changes in VEGF expression in the heart and kidney, using two different age groups of seemingly healthy WR – 6-month-old and 12-month-old. In addition, we report a statistically significant decrease in CD in both ventricles and the kidney as aging advances. Finally, we demonstrated positive correlation between VEGF expression and CD, which were statistically significant in both age groups in the RC and in 12-month-old animals in the two ventricles.

The exact physiological role of VEGF in the heart after embryological development is yet unclear. Giordano *et al*. (2001) performed a heart-specific VEGF knockout experiment which resulted in mice with lower body weight, the density of the wall of their hearts was significantly lower and their hearts were dilated, hypovascularized and with contractile dysfunction, thus highlighting the importance of VEGF for normal cardiac function. The role of VEGF in the

myocardium under pathological conditions, however, has been studied more thoroughly in comparison to its role in physiological aging. Jesmin *et al*. (2005) performed a comparative study on the age-related level of expression of VEGF in the hearts of spontaneously hypertensive rats, stroke-prone spontaneously hypertensive rats, and a control group of Wistar-Kyoto rats (WKY) (Jesmin *et al*., 2005). Their study reported no age-related changes in VEGF expression in the LV of WKY. In contrast, our semi-quantitative data showed an increase in VEGF expression in WR in both LV and RV between 6- and 12-month-old rats. Furthermore, the lack of apparent pathology in the rat model used herein strongly suggests age as the primary contributor to those changes.

In our earlier studies, we measured CD as a marker for statistical evaluation of capillary growth and our results showed an age-related decrease in CD in LV and RV in healthy WR (Iliev *et al*., 2017). The data from the present study confirm those results, as we reported a statistically significant decrease in CD in both ventricles between the two age groups. As previously shown, such decrease in CD leads to impaired microcirculation, which was recently proposed as one of the main factors behind the progressive nature of aging (Jin, 2019). Several factors may influence the decrease in CD in the aging heart. An increase in reactive oxygen species (ROS) with age leads to endothelial cell apoptosis and capillary rarefaction (Yoshida *et al*., 2022). In addition, aging leads to an increase in the so-called 'decoy' receptors for VEGF-A in the blood circulation, which in turn suppresses VEGF-A-mediated neovascularization (Yoshida *et al*., 2022). This may be one explanation for the higher expression of VEGF in older animals, reported herein, which was not mirrored by a similar increase in CD.

Studies on the complex relationship between VEGF and CD have yielded contrasting conclusions. Some authors described no changes in the expression of VEGF while in the same time reporting a significantly lowered CD (Gavin *et al*., 2005). Others established a correlation between the progressive decrease of CD with age and the lowered expression of VEGF (Ahluwalia *et al*., 2010). In the present study, we reported a positive but not statistically significant correlation in the LV and RV of 6-month-old WR and a statistically significant positive correlation in 12-month-old animals. This correlation proves the strong relationship between VEGF expression and CD, however does not explain the contrasting changes in both markers. What these changes likely suggest is that a compensatory increase in VEGF expression by cardiomyocytes takes place as a consequence of the decreased CD in the older experimental group in an attempt to counter capillary rarefaction and prevent the onset of heart failure.

In the kidney, our results for VEGF expression differed from those in the myocardium, as we described a decrease in its expression between the two age groups in both the RC and RM, which confirms the previous literature data (Kang *et al*., 2001). Comparing the two age groups, a statistically significant decrease in CD was reported. It has been demonstrated that tubulointerstitial fibrosis, as a major feature of renal aging, is accelerated by the loss of peritubular CD (Thomas *et al*., 1998; Weinstein & Anderson, 2010). Furthermore, the data obtained in the present work show a positive correlation between the decreased expression of VEGF and the lower CD which is on par with the literature data (Kang *et al*., 2001). Indeed, podocytes and tubular epithelial cells are known to undergo a series of changes as aging advances, but the possible connection between them and the lower levels of VEGF and therefore the decrease in CD has not been fully explored (Camici *et al*., 2011; Susnik *et al*., 2015). It appears that those cells, which are the main source of VEGF in the kidney, fail to support the needed levels of VEGF which triggers a decrease in CD, followed by an acceleration of tubulointerstitial fibrosis.

There were several limitations to the present study which should be noted. Firstly, the expression of VEGF in the heart and kidney was assessed semi-quantitatively and a separate statistical analysis of quantified values of the strength of expression was thus not possible. Secondly, significant inter-observer variation has been noted in the visual quantification of slides processed for immunohistochemistry. In order to resolve this issue, we used an automated software, which eliminates inter-observer discrepancies in the visual assessment. Thirdly, the study only included male WR in order to avoid a possible impact of female sex hormones during the cyclical changes observed in female WR. Finally, representative fields selected for analysis depended on the quality of the obtained specimens and were limited to a certain extent in the hearts and kidneys of younger animals, where parts of the histological material were damaged as part of the routine tissue processing.

In conclusion, our study confirms that VEGF expression is tightly related to CD and therefore plays a crucial role in the maintenance of physiological microcirculation. The decrease in CD mirrored by a higher VEGF expression in the myocardium reveals that an 'uncoupling' of this relationship may represent one aspect of aging in the heart that potentially impairs its function. On the other hand, our observations on the aging kidney revealed that VEGF expression decreases together with CD but the correlation between the two was statistically significant only in the RC. These findings suggest that further studies are needed to fully unravel the mechanisms behind microcirculatory alterations as a key aspect in physiological aging.

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ILIEV, A.; KOTOV, G.; STAMENOV, N.; LANDZHOV, B.; KIRKOV, V.; GAYDARSKI, L. & STANCHEV, S. Cambios microcirculatorios como sello distintivo del envejecimiento en el corazón y riñón. *Int. J. Morphol., 41(2)*:333-342, 2023.

RESUMEN: Los cambios en la microcirculación de múltiples tejidos y órganos se han implicado como un posible mecanismo en el envejecimiento fisiológico. En particular, el factor de crecimiento endotelial vascular es una proteína secretora responsable de regular la angiogénesis mediante la alteración de la proliferación endotelial, la supervivencia, la migración, la degradación de la matriz extracelular y la permeabilidad celular. El objetivo del presente estudio fue evaluar el papel del factor de crecimiento del endotelio vascular en la progresión de las alteraciones morfológicas causadas por el envejecimiento fisiológico en el corazón y riñón y examinar su relación con los cambios en la densidad capilar. Utilizamos dos grupos de ratas Wistar sanas: 6 y 12 meses de edad. La expresión del factor de crecimiento del endotelio vascular se examinó mediante inmunohistoquímica e inmunofluorescencia y se evaluó semicuantitativamente. Los cambios en la densidad capilar se evaluaron estadísticamente y se correlacionaron con la expresión del factor de crecimiento del endotelio vascular. Informamos una inmunorreactividad más fuerte para el factor de crecimiento endotelial vascular en el ventrículo izquierdo en comparación con el derecho y también observamos un aumento en su expresión en ambos ventrículos en animales mayores. Se informaron resultados contrastantes para la corteza renal y la médula. La densidad capilar disminuyó estadísticamente en todas las estructuras examinadas a medida que avanzaba el envejecimiento. Las correlaciones estudiadas fueron estadísticamente significativas en los dos ventrículos en animales de 12 meses y en la corteza renal de ambos grupos de edad. Nuestros resultados arrojan luz sobre algunos cambios en la microcirculación que tienen lugar a medida que avanza el envejecimiento y probablemente contribuyan a un deterioro en la función de los órganos examinados.

PALABRAS CLAVE: Factor de crecimiento endotelial vascular (VEGF); Densidad capilar (CD); Miocardio; Riñón; Envejecimiento.

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